UTILISING CRISPR/Cas 9 FOR GENE EDITING TO ADDRESS HEREDITARY MOVEMENT DISORDER

Abstract

Gene therapy offers a promising approach for addressing hereditary movement disorders like Huntington's disease, Ataxia, Dystonia and Parkinson's disease. A method known as genome editing, involves the modification of DNA by inserting, deleting, or replacing specific sequences, has gained much attention in the past few years. A prominent tool in this field is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) system. Cas9 is obtained from the bacteria, *Campylobacter jejuni*. CRISPR-Cas was originally a part of the *Streptococcus pyogenes* adaptive immune system, but CRISPR/Cas9 now serves as a valuable tool for genome editing, causing breaks in the DNA strands under the guidance of RNA (gRNA). In contrast to alternative genome editing techniques like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 stands out for its potential clinical applicability due to its convenient in vivo delivery. It is highly specific and cuts only a limited number of sites in the genome. This chapter explores and assesses the viability of CRISPR/Cas9 in the preclinical research and its potential use in gene therapy for hereditary movement disorders.

Keywords: CRISPR-Cas9, gene therapy, gene editing, hereditary movement disorders

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I. INTRODUCTION

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the Cas (CRISPR-associated proteins) technology has emerged as a groundbreaking gene-editing tool over the past decade, enabling scientists to precisely and efficiently alter the DNA sequences. This chapter delves into the multifaceted aspects of CRISPR-Cas9 technique that have revolutionized the fields of genetics, biotechnology, and medicine. This technology provides a robust framework for conducting genome editing, enabling researchers to explore and investigate a wide array of diseases (Cox et al., 2015; Jinek et al., 2013; Mali et al., 2013).

CRISPR-Cas9 was initially identified as a peculiar DNA sequence in bacteria, but, when paired with CRISPR-Cas proteins, this system forms a natural defense mechanism used by eubacteria and archaea for protection against invading viruses and plasmid. The Cas9 protein, in particular, acts as molecular "scissors," which is an RNA-guided DNA endonuclease enzyme and is now being utilised as a genome editing tool to induce double-strand breaks (DSB) in DNA. According to Horvath et al., 2010, this system is crucial for acquiring immunity necessary to defend against invading viruses and plasmids. In 1987, researchers discovered CRISPR in the genome of *Escherichia coli*. They identified it as a sequence of 29 nucleotides that repeated several times, interspaced with variable sequence fragments of 32 (Ishino et al., 1987).

Genome editing, a form of genetic engineering, involves the precise modification of DNA through the insertion, deletion or replacement in the genome using nucleases which enable precise modification of genes by introducing DSBs at the target location in the genome. Nucleases that can be used in genomic editing include zinc-finger nuclease (ZFNs) and transcription activator-like effector nuclease (TALENs) which create site-specific DSBs at the target locations. One particularly significant tool in genome editing for this purpose is the CRISPR-Cas9, which is an RNA-guided engineered nuclease (RGEN) system, which has synthetic guide RNA (gRNA) that introduces a DSB at a specific location in the target genome (Barrangou et al., 2007; Garneau et al., 2010). CRISPR-Cas is associated with the adaptive immune system of *Streptococcus pyogenes*. Notably, it offers distinct advantages in clinical applicability compared to other editing technologies.

In medicine, CRISPR-Cas9 holds immense promise for treating genetic disorders. By editing disease-causing mutations, researchers aim to correct the genetic defects at the root level. This technology has the potential to pave the way for ground-breaking therapies, offering hope to patients suffering from previously incurable genetic conditions.

II. WHY IS CRISPR-CAS APPLICABLE FOR GENETIC DISORDERS?

It is widely recognized that genetic diseases, resulting from alterations in DNA sequences, play pivotal roles in various biological systems. In the context of pathogenesis, numerous genes have been identified as crucial contributors to the development of genetic-based diseases. Typically, the correction of defective genes within diseased cells can be achieved through two primary approaches: ex vivo and in vivo interventions.

In ex vivo procedures, diseased cells are extracted, manipulated as necessary using programmable nucleases, and subsequently reintroduced into the original host. Conversely, in vivo therapy involves the direct delivery of modified editing tools, carrying the corrected gene segment, into the body. Hence, these identified genes hold substantial potential as targets for modified nucleases, such as the CRISPR/Cas9 system, aimed at removing or repairing faulty genes, thus paving the way for the development of therapeutic strategies to combat genetic disorders. It's important to note that each programmable nuclease possesses its unique set of advantages and disadvantages, and they are employed in diverse manners to address specific disorders. Various gene-editing techniques have been applied in cell lines, disease models, and even human subjects, as documented by Pandey et al. in 2017.

III. MECHANISM OF ACTION OF CRISPR-Cas9 SYSTEM IN GENE EDITING

CRISPR-Cas was first hypothesized in 2005 (Bolotin et al., 2005; Mojica et al., 2005; Pourcel et al., 2005) and then demonstrated by Barrangou in 2007 (Figure 1). In this system, the CRISPR array stores immunological information in the form of "spacer". The short DNA sequence from invading pathogens are interleaved with the CRISPR DNA repeats.

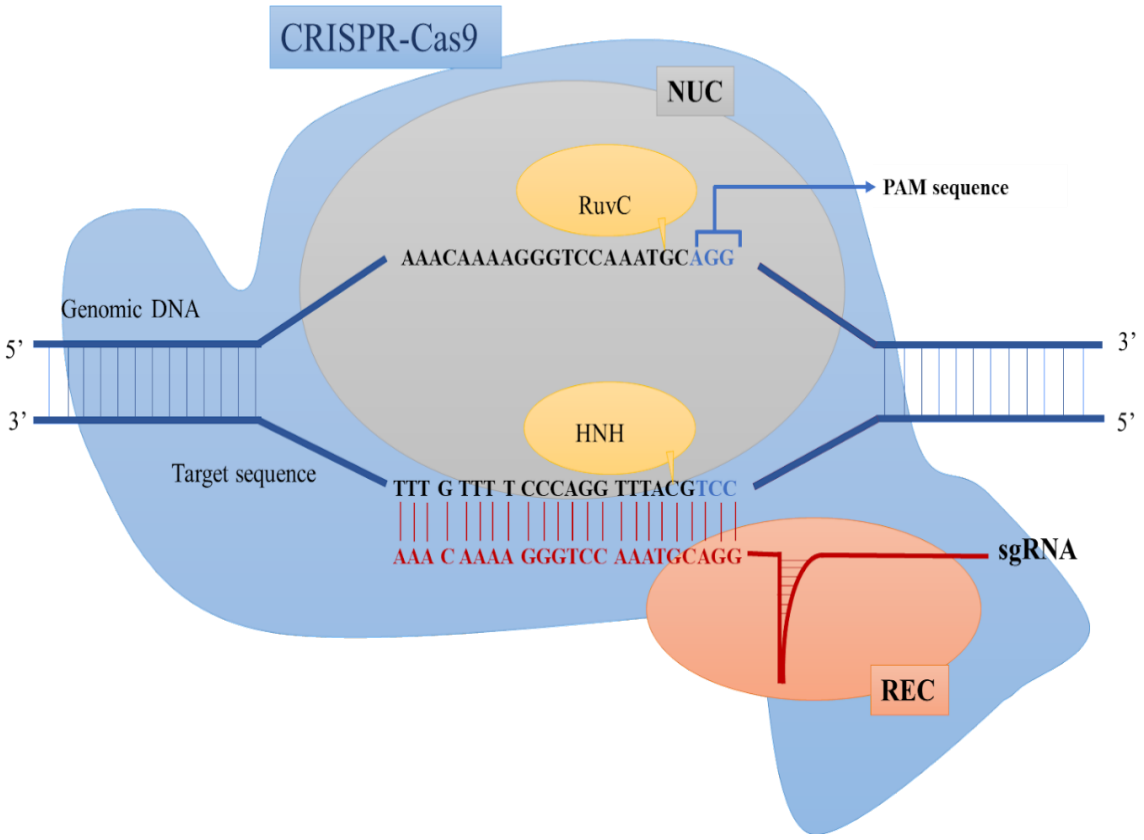

Figure 1: Representing structure of RNA-guided Cas9 nuclease CRISPR-Cas9. It consists of two functional lobes, Cas9 recognition (REC) domain (orange) and nuclease (NUC) lobe (grey). REC lobe is Cas9 recognition domain interacting with sgRNA (red), whereas NUC lobe consists of two nuclease domains (RuvC and HNH) drives the interaction with PAM and target sequence leading to DSB.

The mechanism of action of the CRISPR-Cas9 system, which is classified as RGEN and recognises a specific target sequence of 23 base pairs which is distinct from that of ZFNs and TALENs (Gaj et al., 2013; Deltcheva et al., 2011). Cas9 is used as a nuclease by CRISPR-Cas9, which recognises genomic DNA using gRNA. (Mali et al., 2013; Moscou et al., 2009; Ceasar et al., 2016). The gRNA recognizes approximately 20 base pair nucleotide and requires protospacer adjacent motif (PAM), which recruits Cas9, where Cas9 is guided by a specific sequence of gRNA that is related to trans-activating crRNA (tracrRNA) and forms the complementary DNA (cDNA) target sequence, resulting in site-specific DSB (Garneau et al., 2010; M Jinek et al., 2012; Jiang et al., 2017) (Figure 1).

In the CRISPR-CAS mechanism, two distinct mechanisms exist for repairing DSBs created by the Cas-9 protein: non-homologous end joining (NHEJ) and homology-directed repair (HDR**)** (Figure 2). NHEJ mends DSBs through an enzymatic process, independent of homologous DNA, and operates throughout the cell cycle. It is the predominant and efficient cellular repair mechanism, albeit error-prone, often causing random insertions and deletions at the cleavage site, leading to frameshift mutations or premature stop codons (Yang et al., 2020; Asmamaw et al., 2021).

On the other hand, HDR is highly precise and utilizes a homologous DNA template. This repair mechanism requires a substantial amount of exogenous donor DNA containing the target sequence. HDR achieves precise insertion and replacement by integrating the donor DNA template with sequence homology at the predicted DSB site (Yang et al., 2020; Liu et al., 2019; Asmamaw et al., 2021).

Due to CRISPR-Cas9 ability to simultaneously disrupt several genes, it can be used to research genetic interaction and create models for multigenic disorders. By regulating the short specific part of gRNA, the Cas protein can target particular DNA sequences. One significant issue with Cas is the occurrence of off-target effects, which involve the non-specific recognition and digestion of non-targeted DNA sequences. Cas protein can be dual-guide RNAs or single-guide RNAs. For CRISPR-Cas9 applications to human disease to be successful, the technique for preventing off-target effects needs to be further researched.

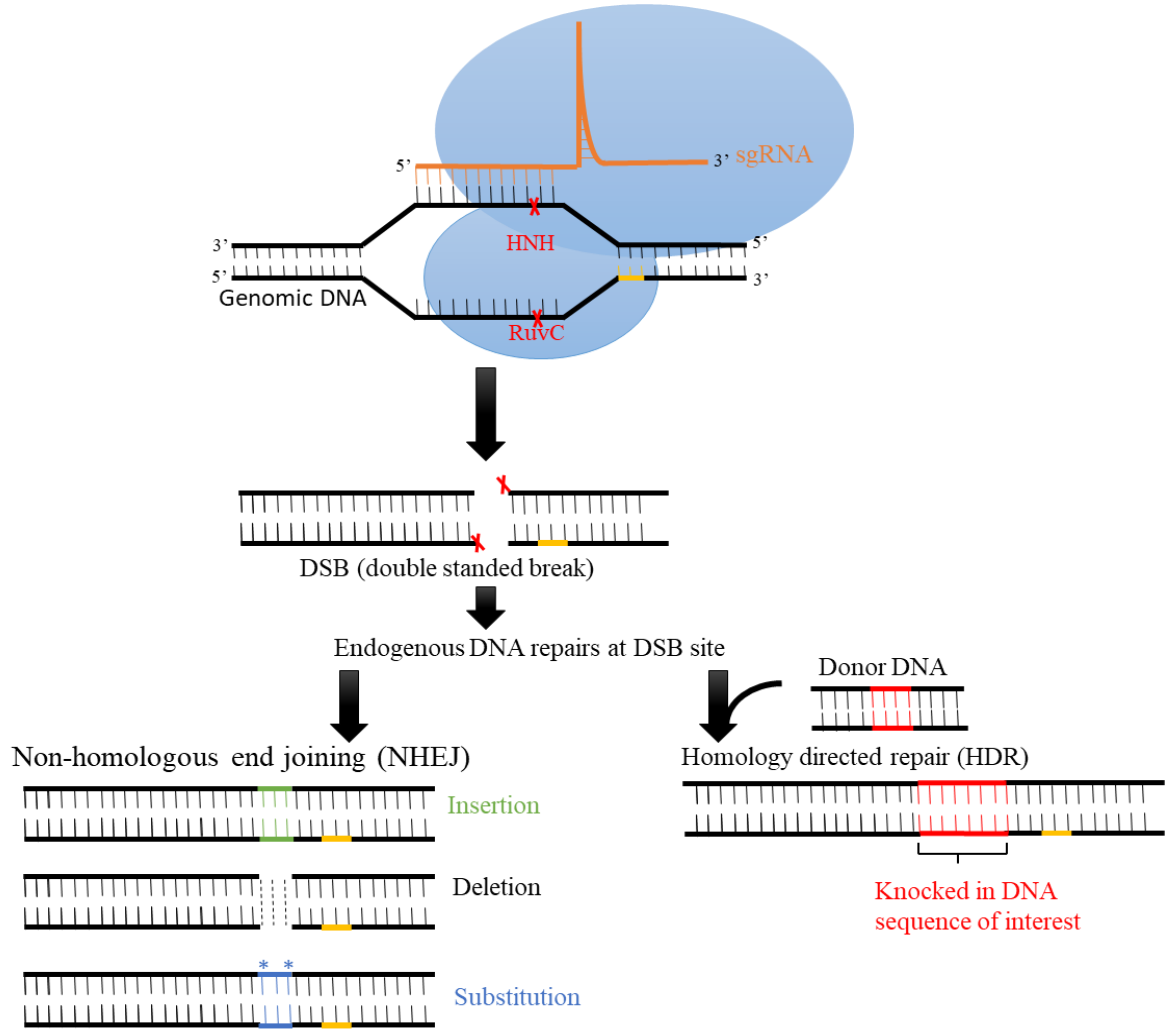

Figure 2: Representing the mechanism of CRISPR-Cas9 mediated genome editing. When Cas9 induces double standed break (DSB), it initiates two repair mechanism such as non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is characterized by its error prone nature and can result in random deletion or insertion at the site of junction. Conversely, the HDR pathway can be used within a repair template, leading to precise genome editing.

IV. CRISPR-Cas9 AS A THERAPEUTIC INTERVENTION FOR HEREDITARY MOVEMENT DISORDER

The pathophysiology of hereditary movement disorders has been associated with a multitude of genes, rendering them viable candidates for the utilisation of the CRISPR-Cas9 system in the development of therapeutic strategies aimed at modifying the course of the diseases (Wooseok Im et al., 2016) . Hereditary movement disorders encompass conditions such as ataxia, dystonia, chorea, spastic paraparesis, Huntington's disease, and Parkinson's disease, among others, all of which manifest due to heightened occurrences of genetic mutations.

Trinucleotide repeat disorders, exemplified by Huntington's disease, are characterized by an elongated polyglutamine region that surpasses a defined threshold. This expansion triggers protein misfolding and aggregate formation, ultimately exerting profound impacts on neuronal cellular and molecular processes (Dickinson et al., 2013]. The prospect of rectifying the anomalous CAG repeat sequence within normal cells or amending mutations within patient-derived cells holds promise through tailored nucleases, as demonstrated in modified nuclease studies (Liu et al., 2005; Freude et al., 2014). Conversely, in the context of Parkinson's disease (PD), the pathological underpinning frequently involves the presence of misfolded protein entities termed Lewy bodies. The central constituent of these aggregates, α-synuclein, significantly contributes to PD pathology. A principal genetic contributor to PD is the Synuclein Alpha (SCNA) gene, responsible for encoding α-synuclein (Karimian et al., 2020). A prevalent genetic anomaly in PD is a mutation within the leucine-rich repeat kinase 2 (LRRK2) gene, culminating in dopaminergic neuron toxicity. By leveraging the precision of CRISPR/Cas9 technology, it becomes conceivable to rectify the LRRK2 mutation, potentially mitigating the incidence of PD within afflicted families. Likewise, spinocerebellar ataxia types 1, 2, 3, 6, 7, and 17, characterized by autosomal dominant inheritance, manifest as trinucleotide repeat disorders in which an abnormal protein with an extended polyglutamine tract accumulates, precipitating cerebellar ataxia (Kardasiewicz et al., 2012). This shared pathogenic mechanism underscores the role of aberrant protein accumulation in neurodegenerative processes. It is noteworthy that while multifactorial etiological factors are implicated in Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, an analogous manifestation of abnormal misfolded proteins is evident.

Some hereditary movement disorder occurs in autosomal recessive patterns caused by the loss-of-function mutation of a particular gene. Genes responsible for hereditary movement disorder can be knocked in a specific transgene by CRISPR-Cas9. Some hereditary movement disorder occurs in autosomal recessive patterns caused by the loss-of-function mutation of a particular gene. Genes responsible for hereditary movement disorder can be knocked in a specific transgene by CRISPR-Cas9.

V. FUTURISTIC TRENDS OF CRISPR-Cas9

The emergence of CRISPR-Cas-mediated genome editing technologies has ushered in a transformative and versatile avenue for the manipulation, control, and observation of genetic material. These technologies are regarded as a significant landmark in 21st-century molecular biology. Thus far, CRISPR-Cas systems have found wide-ranging utility in dissecting gene functions, advancing human genetic therapies, precision-oriented drug creation, establishing animal models, and enhancing livestock breeding. These applications substantiate their immense potential for further advancement.

The evolution of the CRISPR system into a gene-editing tool has sparked a revolutionary shift in the life sciences. The advent of next-generation gene editing technologies has further enhanced the versatility of the CRISPR system, offering researchers potent and innovative tools to explore biological systems and study human diseases. CRISPR technologies hold significant promise as potential treatments for genetic diseases and genetic disorders in humans. Base editing screening has emerged as a valuable method to investigate the connections between gene mutations and their effects. It’s remarkable ability to knock out specific genes without causing extensive chromosomal rearrangements by introducing a

premature stop codon or disrupting the splice site. CRISPR-Cas9 holds the potential for targeted manipulation of aberrant protein synthesis and accumulation, demonstrating efficacy in mitigating the underlying pathology of associated disorders (Rodriguez et al,. 2019). Certain hereditary movement disorders manifest through autosomal recessive inheritance patterns, attributed to loss-of-function mutations within specific genes. The genetic loci accountable for these hereditary movement disorders can be precisely integrated into a designated transgene via CRISPR-Cas9 technology, offering a mechanism for functional restoration.

Recent findings indicate that the CRISPR-Cas system exhibits a higher efficiency in-vitro compared to in vivo. This intriguing disparity suggests a promising avenue for expediting medical research through the strategic utilisation of genetically modified cell models. Such an approach has the potential to significantly reduce the time required for research endeavors. To date, researchers have effectively harnessed CRISPR-Cas systems to carry out precise genetic modifications across diverse cell lines. These cell types encompass a range from tumor cells to mature adult cells, as well as versatile stem cells. This versatile application allows for the emulation of a diverse array of human diseases, thereby facilitating a deeper understanding of their underlying mechanisms. Furthermore, this technique paves the way for the exploration of innovative therapeutic approaches. Nonetheless, practical utilization of CRISPR-Cas systems presents extant challenges that necessitate resolution. Vigorous endeavors are imperative to ascertain enduring safety and efficacy, mandating meticulous evaluation and ongoing study.

REFERENCE

[1] Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. Science (New York, N.Y.), 327(5962), 167–170. https://doi.org/10.1126/science.1179555
[2] Cox, D. B., Platt, R. J., & Zhang, F. (2015). Therapeutic genome editing: prospects and challenges. Nature medicine, 21(2), 121–131. https://doi.org/10.1038/nm.3793
[3] Jinek, M., East, A., Cheng, A., Lin, S., Ma, E., & Doudna, J. (2013). RNA-programmed genome editing in human cells. eLife, 2, e00471. https://doi.org/10.7554/eLife.00471
[4] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.), 337(6096), 816–821. https://doi.org/10.1126/science.1225829
[5] Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. Journal of bacteriology, 169(12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987
[6] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–1712.
[7] Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010;468:67–71.
[8] Gaj, T., Gersbach, C. A., & Barbas, C. F., 3rd (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in biotechnology, 31(7), 397–405. https://doi.org/10.1016/j.tibtech.2013.04.004
[9] Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature, 471(7340), 602–607. https://doi.org/10.1038/nature09886
[10] Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. Science (New York, N.Y.), 339(6121), 823–826. https://doi.org/10.1126/science.1232033

[11] Moscou, M. J., & Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. Science (New York, N.Y.), 326(5959), 1501. https://doi.org/10.1126/science.1178817
[12] Liu P-Q, Tan S, Mendel MC, Murrills RJ, Bhat BM, et al. (2005) Isogenic human cell lines for drug discovery: Regulation of target gene expression by engineered zincänger protein transcription factors. J Biomol Screen 10: 304-313.
[13] Freude K, Pires C, Hyttel P, Hall V (2014) Induced pluripotent stem cells derived from Alzheimer's disease patients: He promise, the hope and the path ahead. J Clin Med 3: 1402-1436.
[14] Kordasiewicz, H. B., Stanek, L. M., Wancewicz, E. V., Mazur, C., McAlonis, M. M., Pytel, K. A., Artates, J. W., Weiss, A., Cheng, S. H., Shihabuddin, L. S., Hung, G., Bennett, C. F., & Cleveland,
[15] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.), 337(6096), 816–821. https://doi.org/10.1126/science.1225829
[16] Dickinson, D., Ward, J., Reiner, D. et al. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. Nat Methods 10, 1028–1034 (2013). https://doi.org/10.1038/nmeth.2641
[17] Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S. Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta Mol Cell. 2016;1863(9):2333–2344. doi:10.1016/j.bbamcr.2016.06.009
[18] Pandey, V. K., Tripathi, A., Bhushan, R., Ali, A., & Dubey, P. K. (2017). Application of CRISPR/Cas9 Genome Editing in Genetic Disorders: A Systematic Review Up to Date. In Journal of Genetic Syndromes & Gene Therapy (Vol. 08, Issue 02). OMICS Publishing Group. https://doi.org/10.4172/2157-7412.1000321
[19] Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017;46(1):505–529. doi:10.1146/annurev-biophys-062215-010822
[20] Yang H, Ren S, Yu S, et al. Methods favoring homology-directed repair choice in response to CRISPR/cas9 induced-double strand breaks. *Int J Mol Sci*. 2020;**21**(18):1–20. doi: 10.3390/ijms21186461
[21] Liu M, Rehman S, Tang X, et al. Methodologies for improving HDR efficiency. *Front Genet*. 2019;**9**:1–9. doi: 10.3389/fgene.2018.00691
[22] Misganaw Asmamaw & Belay Zawdie (2021) Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing, Biologics: Targets and Therapy, 15:, 353-361, DOI: 10.2147/BTT.S326422
[23] Karimian, A., Gorjizadeh, N., Alemi, F., Asemi, Z., Azizian, K., Soleimanpour, J., Malakouti, F., Targhazeh, N., Majidinia, M., & Yousefi, B. (2020). CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases. In Life Sciences (Vol. 259, p. 118165). Elsevier BV. https://doi.org/10.1016/j.lfs.2020.118165
[24] Im, W., Moon, J., & Kim, M. (2016). Applications of CRISPR/Cas9 for Gene Editing in Hereditary Movement Disorders. Journal of movement disorders, 9(3), 136–143. https://doi.org/10.14802/jmd.16029
[25] Rodríguez-Rodríguez, D. R., Ramírez-Solís, R., Garza-Elizondo, M. A., Garza-Rodríguez, M. L., & Barrera-Saldaña, H. A. (2019). Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review). International journal of molecular medicine, 43(4), 1559–1574. https://doi.org/10.3892/ijmm.2019.4112
[26] Shao M, Xu T, Chen C. The big bang of genome editing technology: development and application of the CRISPR/CAS9 system in disease animal models. Sci Press Zool Res. 2016;37(2):1–11.

